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New Generation Vaccines

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Liposomes as Adjuvants for Vaccines

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I. INTRODUCTION

Numerous reviews of liposomes as carriers of antigens and adjuvants have been published since 1990 [1-7]. Liposomes have the advantage of being biodegradable vehicles that mimic the lipid structure of natural lipid bilayer membranes. Because of this, insoluble membrane antigens can often be reconstituted in a form that exposes purified antigenic epitopes, such as purified viral antigens, in conformations that are presumed to be similar to those found in the original organism [8,9]. Liposomes are also avidly ingested by phagocytes, cells then serving as antigen-presenting cells [10-12]. In addition to these natural attributes, liposomes also have the convenient characteristic of encapsulating soluble antigens and then serving as permeability barriers for slow release of the antigens. Besides antigens, additional adjuvants, such as lipid A (the lipid moiety of gram-negative bacterial lipopolysaccharide) or lipophilic muramyl dipeptide (MDP) derivatives and analogs, can be incorporated either into the lipid bilayer or encapsulated (if soluble) in the aqueous regions [3,6].

A. Safety of Adjuvants

Safety, and the perception of safety, of adjuvant formulations remains one of the most important considerations in the development of novel adjuvants [13-16]. A dramatic example of the role of safety comes from the experience with incomplete Freund's adjuvant (IFA), a potent water-in-oil adjuvant formulation. More than 150,000 persons in the United States, and more than a million people worldwide, were successfully administered vaccines containing IFA as a constituent in the 1950s and 1960s [17]. This

experience would suggest that IFA could fulfill the pressing worldwide need, particularly among developing nations, for a useful and inexpensive generic adjuvant. Unfortunately, although several retrospective studies have not found any increased incidence of cancer, autoimmune diseases, or any other apparent long-term toxicity in vaccinees [18-20], 40 localized cystic reactions requiring surgical intervention among 900,000 vaccinees in the United Kingdom in the 1960s caused abandonment of large-scale applications of IFA [17]. Despite a recommendation in 1968 for resumption of widespread use of IFA in the United States [17], the perception, whether appropriate or not, that IFA might be unsafe has burdened IFA with a difficult and tarnished historical reputation that may raise hurdles for ultimate acceptance by users and vaccine regulatory agencies. The historical lesson of IFA, in which local cystic reactions prevented commercial acceptance of a potent vaccine adjuvant, has been a unique motivating force that has driven vaccine developers to achieve safety optimization of modern adjuvants, including liposomal adjuvant formulations.

In the course of studying the preclinical safety of an influenza vaccine containing IFA, more than seventy monkeys were studied histologically and serologically for more than a year, and local reactions were minimized or eliminated by appropriate refinement of the IFA constituents [17]. Recently, the onerous problem of preclinical testing of novel experimental adjuvants, particularly those that have been previously tested in a phase I trial with a different antigen in humans, has been ameliorated in the United States by productive communication between vaccine makers and the Food and Drug Administration, resulting in considerable simplification and some degree of standardization of phase I preclinical safety testing [21].

Table 1 Induction of Cytotoxic T Lymphocytes by Liposome-Encapsulated Antigen

| Antigen | Liposome composition | Reference |
|--|---|--------------------------|
| In vitro studies | | |
| MHC antigens (H-2 in mice) | Egg PC/CHOL (70:30 w/w) | Hale [29] |
| MHC antigens (H-2 in mice) | Egg PC/CHOL (70:30 w/w) | Hale and McGee [30] |
| Human colon tumor antigens | PC/CHOL/PA (7:2:1) | Raphael and Tom [31, 32] |
| LS174T colon tumor cells | | |
| Ovalbumin | DOPC/DOPS (4:1) and DOPE-PHC (4:1) | Harding et al. [33] |
| Murine hemoglobin | | |
| Bovine ribonuclease | | |
| Hen egg lysozyme | | |
| Ovalbumin | DOPE/DOSG (1:1) and DOPC/PS/CHOL (5:2:3) | Reddy et al. [34] |
| Ovalbumin | DOPE/DOSG (1:1) and DOPC/PS/CHOL (5:2:3) | Zhou et al. [35] |
| In vivo studies | | |
| Haemagglutinin neuraminidase | MDP and MDP/CHOL (1:1 w/w) | Nerome et al. [36] |
| Ovalbumin β -galactosidase | DOPE/DOSG (1:1) and DOPC/PS/CHOL (5:2:3) | Reddy et al. [37] |
| Ovalbumin | DOPE/DOSG DOPE/DPSG DOPE/CHEMS DOPC/DOPS (all 4:1) \pm 50 μ g lipid A | Collins et al. [38] |
| Ovalbumin | DPPC/DPPG/CHOL (9:1:8) \pm 50 μ g lipid A | |
| Ovalbumin | PC/lysoPC/CHOL (6.9:0.1:3 neutral) PC/lysoPC/SA/CHOL (6.9:0.1:1:2 positive) PC/lysoPC/DCP/CHOL (6.9:0.1:1:2 negative) | Lopes and Chain [39] |
| Multiple antigen peptide system (MAPS) from GP120 of HIV-1 (B2M-P3C) | Egg PC/CHOL/SA (7.5:1:0.25 w/w) | Defoort et al. [40] |
| Glycoprotein B from HSV | Cationic lipids (dioleoyloxypropyl-trimethylammonium methyl sulfate DOTAP) | Walker et al. [41] |
| Ovalbumin | Commercially available DOTAP | Chen et al. [42] |
| Repeatless <i>P. falciparum</i> CS protein | DMPC/DMPG/CHOL/lipid A (0.9:0.1:0.75:0.026) | White et al. [43] |
| SIV gag protein-derived peptide (p11C) | PS/CHOL (9:1)-envelope glycoproteins and lipids of Sendi virus | Miller et al. [44] |
| SIV gag protein-derived peptide (M90-07A) | DMPC/DMPG/CHOL/lipid A (0.9:0.1:0.75:0.1) | Yasutomi et al. [45] |

Key: CHEMS, cholesterol hemisuccinate; CHOL, cholesterol; CS, circumsporozoite; DCP, dicetyl phosphate; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; DOPC, dioleoyl phosphatidylcholine; DOPE, dioleoyl phosphatidylethanolamine; DOPS, dioleoyl phosphatidylserine; DOSG, 12-dioleoyl-sn-3 succinylglycerol; DPPC, dipalmitoyl phosphatidylcholine; DPPG, dipalmitoyl phosphatidylglycerol; DPSG, dipalmitoyl succinylglycerol; lysoPC, lysophosphatidylcholine; MDP, muramyl dipeptide; MHC, major histocompatibility antigen gene complex; PA, phosphatidic acid; PC, phosphatidylcholine; PHC, palmitoyl homocysteine; PS, phosphatidylserine; SA, stearylamine.

II. SAFETY AND POTENCY OF LIPOSOMAL VACCINES

Liposome formulations, including liposomes containing extremely high concentrations of potentially dangerous endotoxic lipid A, have regularly and routinely

passed the current preclinical safety tests in the United States and have shown little reactogenicity in human trials [22]. Several unpublished phase I trials, using three different antigens—two malaria antigens and one human immunodeficiency virus (HIV) antigen—currently involving more than a hundred volunteers,

Table 2 Protective Liposomal Vaccines in Animal Disease Models

| Disease or etiologic agent | Antigen | Route of immunization | Animal | Reference |
|---|---|-----------------------|-----------------------------|------------------------------|
| Malaria | Killed <i>Plasmodium falciparum</i> merozoite antigen | IM | Owl monkey | Siddiqui et al. [46,47] |
| Malaria | Multiple antigen peptide from <i>Plasmodium yoelii</i> | SC | BALB/cByJ mice | Wang et al. [48] |
| <i>Streptococcus pneumoniae</i> | circumsporozoite protein | IP, or IV | BALB/c mouse | Snippe et al. [49] |
| Epstein-Barr virus-induced lymphoma | Hexasaccharide-lipid conjugate | IP | Cotton-top tamarin | Epstein et al. [50] |
| Rabies virus | MA gp340 | IM | Hamster | Perrin et al. [51] |
| Snake venom | Glycoprotein | SC | TFW mouse | New et al. [52] |
| Carpet viper | Whole venom | IV | Sheep | Theakston et al. [53] |
| Carpet viper | Whole venom | SC or IV | TFW mouse | Freitas et al. [54] |
| Rattlesnake | Toxic fraction | SC | C57B1/6 mouse | Chaves-Olortegui et al. [55] |
| Scorpion toxin | | | | |
| Dental caries induced by: <i>Streptococcus sobrinus</i> | Ribosomal protein | PO | Germ-free rat | Gregory et al. [56] |
| Dental caries induced by: <i>S. mutans</i> | Anti-idiotypic antibodies | PO | Germ-free rat | Jackson et al. [57] |
| <i>S. mutans</i> | Serotype-specific carbohydrate | PO | Germ-free rat | Michalek et al. [58] |
| Melanoma | B16 tumor-associated antigens | SC | C57B1/6 mouse | Phillips et al. [59] |
| Influenza A virus | Free inactivated virus (mixed with liposomes containing IL-2) | IP | BALB/c mouse | Mbawuikwe et al. [60] |
| Herpes simplex virus | Glycoprotein D | IC | Guinea pig (female Hartley) | Ho et al. [61,62] |
| Herpes simplex virus | Glycoprotein D | IP | C3H/HeN mouse | Brynstad et al. [63] |
| Herpes simplex virus | Free glycoprotein D (mixed with liposomes containing IL-2) | SC | Guinea pig (female Hartley) | Ho et al. [64] |
| <i>Leishmania major</i> (cutaneous leishmaniasis) | Lipophosphoglycan and gp63 | IV | BALB/c mouse | Kahl et al. [65] |
| <i>Toxoplasma gondii</i> | Membrane antigen p30 | IP | Swiss-Webster mouse | Bülow and Boothroyd [66] |
| Thymoma (transfected with ovalbumin) | Ovalbumin | IV | C57BL/6 mouse | Zhou et al. [67] |
| Simian immunodeficiency virus | Four SIV envelope- β -galactosidase fusion proteins | IM | Rhesus macaque | Alving et al. [68] |

have now supported the previous observation of minimal or absent systemic or local reactogenicity of an aluminum hydroxide-adsorbed liposomal adjuvant formulation containing high concentrations of lipid A [22]. The liposomal formulation has consistently induced very high levels of antibodies, surpassing the immunostimulating capabilities of many other adjuvants. Although liposomes induce strong specific lymphoproliferative responses in humans, in two instances where they have been examined, the liposomes have not yet succeeded (nor have other adjuvants) in causing any substantial induction of circulating cytotoxic T lymphocytes (CTLs).

A liposomal hepatitis A vaccine manufactured in Switzerland has been found to have an excellent record of safety and potency and has recently been commercially licensed in Switzerland [9,23–27]. As compared with aluminum hydroxide adsorption of the antigen, the liposomal hepatitis A vaccine exhibited significantly reduced pain at the site of injection in volunteers [9,24]. Very high titers of antibodies were induced by a single injection of the vaccine [24].

When tested in nursing home patients, a liposomal influenza vaccine containing hemagglutinins from three strains of influenza provided the highest geometric mean titers, the highest rates of fourfold or more increases of titer, and the highest rates of protective titers against influenza as compared with commercial whole-virus vaccine and subunit vaccines [26].

A multiantigen "super" combination vaccine is being planned that will contain hepatitis A antigen, hepatitis B surface antigen, diphtheria, alpha- and beta-tetanus, as well as hemagglutinin and neuraminidase from three different influenza strains [27]. Although the phenomenon of "antigenic competition" did cause some interference between antigens in this complex vaccine, this was largely or completely overcome by changing the ratios of constituents.

A liposomal vaccine containing hemagglutinin and neuraminidase antigens from influenza was tested in a phase I trial in Japan [28]. The liposomal adjuvant formulation included a lipophilic MDP constituent (B30-MDP). When compared with three hemagglutinin vaccines that were currently being used, the liposomes induced higher antibody titers against two strains and lower titers against the third. The formulation exhibited some degree of systemic and local reactogenicity that was attributed at least in part to the B30-MDP.

III. GENERATION OF CYTOTOXIC T LYMPHOCYTES

Liposomes have been demonstrated by many laboratories to have considerable potency, both in vitro and in vivo as adjuvant formulations for induction of CTLs in experimental animal models. Table 1 summarizes many of the studies that have been published in this area.

IV. ANIMAL MODELS OF PROTECTIVE IMMUNITY

Although liposomes have been successfully applied as potent immunostimulating agents for vaccines in humans, numerous additional potential vaccine applications could be developed. The scope of potential vaccine applications has been demonstrated with appropriate animal models. Table 2 summarizes many of the published reports in which putative liposomal vaccines have been tested for the ability to induce protective immunity against experimental challenge with the appropriate disease-causing agent. The range of possible applications provides considerable optimism that the liposomal platform will be a useful adjunct to the development of a broad spectrum of modern vaccines.

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